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SUBSTITUTE SPECIFICATION AND ABSTRACT

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FLUORESCENCE MICROSCOPE

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Fig. 1 shows the beam path in a microscope equipped for fluorescence applications. The light from an additional light source (1) passes through a heat-absorbing filter (2), red attenuating filter/stop slide (3) and a field diaphragm (4) to the excitation filter (5). The latter is installed in the reflector slide of the microscope which also contains a dichroic beam splitter (6). The dichroic beam splitter reflects the shortwave excitation light through the objective (7) into the specimen or preparation (8).

The occurring emission is collected by the objective (7) and - because it has greater wavelengths than the excitation light - is passed by the dichroic beam splitter (6). The beams now pass through the emission filter (9). The remainder of the excitation light is filtered out by the latter. For this reason, this filter is also referred to as a blocking filter. As is conventional, the tube lens (10) and eyepiece (11) form the microscope image formed of fluorescent light.

In order to avoid image offset (pixel shift), multiple exposures in fluorescence recordings with different emission filter sets (A, B) require an optimal congruence of the object image in the individual recordings. However, there are technological limits in this respect.

Because of the different wedge angles of the emission filters (A_{Em} , B_{Em}) and of the color splitters, the filter combinations needed for the fluorescence application cause a slight image offset. This is shown in Fig. 2.

The reference symbols have the following meanings:

- A_{Em} emission filter of filter set A
- B_{Em} emission filter of filter set B
- a_1 light beam striking A_{Em}
- b_1 light beam striking B_{Em}
- a_2 light beam deflected by A_{Em}
- b_2 light beam deflected by B_{Em}

α_A angle between the incident light beam a_1 and the deflected light beam a_c of filter A_{EM}

α_B angle between the incident light beam b_1 and the deflected light beam b_2 of filter B_{EM}

5 E image plane

$\overline{P_A P_B}$ distance (pixel shift) between the image points impinging on the image plane E

10 The light beams a_1 and b_1 impinge on the emission filters A_{Em} and B_{Em} of the corresponding filter sets A and B. The beam is deflected in more or less opposite directions because of the existing wedge angle of the filters depending on the installed position (a_2 and b_2 are greatly exaggerated in the drawing in order to illustrate the process). Therefore, the image points impinging on the image plane E do not lie exactly one above the other, but are offset relative to one another by the

15 pixel shift. Even with the close tolerances of the filters sets by Carl Zeiss with a slight image offset, this offset still occurs to a slight extent.

According to the invention, as is shown in Fig. 3, the filters are aligned with one another with respect to their wedge angle. The filters are measured and marked by the microscope manufacturer beforehand with respect to wedge

20 angle and orientation, for example, in an autocollimator, e.g., by means of a line S on the side which can be arranged, e.g., on the side located opposite the deflecting direction through the wedge effect. When the filter is inserted into the respective filter module of the microscope, this filter module also has a marking which is made to coincide with the marking on the filter. Identical orientation of the filters is

25 ensured in this way.

After the emission filters A_{Em} and B_{Em} are swiveled in (see Fig. 1), the impinging light beams a_1 and b_1 are deflected in the same direction (a_2 and b_2). In this way, the pixel shift which exists to a slight extent in any case is minimized or, ideally, compensated (pixel shift $\overline{P_A P_B}$ ').

30 In this connection, the wedge angles can also be determined on the part of the manufacturer and filters with identical wedge angles can be marked and correlated by the user.